REMARKS

Reconsideration is requested.

Claims 1-12 have been canceled, without prejudice.

Claims 13-49 have been added. No new matter has been added. Support for the newly added claims may be found throughout the specification and originally-filed claims. Support for the recitations of, for example, claims 18-20 may be found on page 12, line 6 and page 13, lines 26-34 of the specification. Support for the recitations of, for example, claims 25-28 may be found on page 6, lines 13-14 of the specification. Support for the recitations of, for example, claims 29-31 may be found on page 4, line 25-28 of the specification

Attached is a Renewed Petition Under Rule 181 requesting reconsideration of the Decision dated September 23, 2003 and again requesting that the Commissioner invoke his supervisory authority and have the restriction requirement withdrawn, at least insofar as a single primer set and single probe are required to be elected. Consideration and a decision on the attached Renewed Petition Under Rule 181 is requested prior to issuance of a further Action on the merits by the Examiner so that the issues raised in the attached Petition may be considered by the Commissioner and the applicants may have a decision on the same prior to further consideration of the issues raised by the Examiner, and so that the Examiner may consider any further issues raised by the Commissioner prior to the issuance of a further Action.

The applicants respectfully submit that the Decision dated September 23, 2003, contains a number of errors and, at a minimum, correction of the record is believed to be required. Moreover, the Decision fails to address substantive issues requested in the Rule 181 Petition of March 10, 2003, to be considered by the Commission. Consideration of these issues prior to the Examiner's next substantive Action is requested.

As a result of the Decision, the applicants understand that the following summarizes the status of the application:

Incorrect → the Response dated June 24, 2002 has not been entered;

ok → the Office Action mailed September 10, 2002 has been vacated;

ok → the Amendment filed March 10, 2003, has not been entered;

the Examiner improperly withdrew methods of using probes other than SEQ ID NO:15 and primers other than SEQ ID NOs: 18 and 19 from consideration as being directed to non-elected inventions, in the Office Action dated September 10, 2002; and

a response is due to the Office Action mailed May 22, 2002, by October 23, 2003 (i.e., one month from the mail date of the Decision, extendible to March 23, 2004, by filing the requisite extension petition and fee).

As the Office Action mailed September 10, 2002 has been vacated, the restriction requirement has not technically been made final. The Office is requested, in any event, to consider the attached Renewed Rule 181 Petition, as authorized by the Decision, prior to the Examiner mailing a further Office Action on the merits. It has been

the undersigned's experience in this regard that the Office has been reluctant to consider Rule 181 Petitions requesting that the Commissioner invoke his supervisory authority with regard to restriction requirements until after the requirement is made final. The Office is urged, in the interest of efficient prosecution, to consider the attached Renewed Petition, and preferably contact the undersigned to discuss the same if questions arise, prior to the Examiner issuing a further Action on the merits.

The present response, in the form of a further Amendment, to the Office Action dated May 22, 2002, is presented, as required by the Decision. As the Amendment of March 10, 2003, which canceled claims 1-12 and added new claims 13-29, has not been entered, as described in the Decision, the present Amendment presents new claims numbered from new claim 13. The Office is requested to advise the undersigned if the new claims of the present Amendment are to be added as new claims numbered from claim 30 (i.e., the first new claim after the last added claim of the unentered Amendment).

The applicants request reconsideration of the Decision based on at least the following errors and deficiencies in the Decision mailed September 23, 2003:

(1) The Decision erroneously concludes that the election of March 14, 2002 was "incomplete, in as far as no primer sets were elected to correspond with probes detecting" the organisms of the elected probes. See, page 2, 4th full ¶ and page 4, lines 7-9 ("specific primer sets and their corresponding probes for each of the organisms listed in Tables 3, 4 and 5 were not elected") of the Decision.

(2) the Decision erroneously concludes that the applicants Amendment dated June 24, 2002 "again failed to elect one **set** for [sic, of] primers for each of the required regions in Tables 2 and 4." See, first full ¶ of page 3 and page 4, 2nd full ¶ ("without an election of the specific primers needed to amplify out the various gene regions, the election of the specific probe was insufficient") of the Decision.

(3) The Decision fails to indicate where the PCT rules relating to unity of invention allow for a separate requirement to elect one set of primers and a probe within the requirement to elect between methods (i.e., the Examiner's Group I) and "primers, probes and kits (i.e., the Examiner's Group II). See, page 2 of the Office Action dated May 22, 2003 and page 2 of the Office Action dated January 15, 2002, wherein the Examiner indicated the election of a primer and probe was "necessary because the primers and probes are structurally unique and are used for functionally different purposes of amplifying and/or detecting structurally and functionally unrelated products" and page 3 of the Office Action dated September 10, 2002, wherein the Examiner indicated that a serious search burden would be presented if all of the primers and probes were searched because "there are only two sequence processors in the entire [Patent Office] technology center." See also, the applicants requests of March 10, 2003, page 3 of the Rules 181 Petition, June 24, 2002, page 2 of the Response, and March 14, 2002, pages 1 and 2 of the Response, wherein the applicants have requested a basis in the PCT Rules relating to unity of invention for the further restriction requirement with regard to election of a specific primer set and a probe. Moreover, the statement in the Decision that "typically only one set of primer [sic, primers] and probe combinations will be examined within a single patent application.

See MPEP 803.01 which is directed to the examination of sets of molecules" (see, page 3, penultimate paragraph, of the Decision) is, with due respect, inadequate to support a finding of lack of unity of invention in a U.S. national phase of a PCT application.

(4) The Decision improperly considered the Amendment of March 10, 2003, to be "non-responsive" based on the Commissioner's view that dependent claim 19 of the Amendment is unclear. See, the whole of pages 4-5 of the Decision.

Consideration of the following with regard to the applicants previous election (i.e., points **(1)** and **(2)** above), is requested.

The Commissioner and Examiner are urged to appreciate that the Examiner required election of "one primer from Tables 2 and 4 for each of the required regions" and selection of "one primer set from Tables 2 and 4 for each of the required pathogens." See, passages quoted on pages 1 and 2 of the Decision.

The "required regions" and "required patogens" are repeated in the passage spanning pages 2-3 of the Decision. Specifically, the pathogens of the claims are as follows (with the corresponding regions in parentheses) :

Enterovirus (i.e., "the 5'noncoding region for enterovirus" of claim 1)

Influenza A (i.e., "the non-structural protein gene from influenza A" of claim 1)

Influenza B (i.e., "the non-structural protein gene from influenza B" of claim 1)

Adenovirus (i.e., "the hexon gene for adenoviruses" of claim 1)

Parainfluenza 1 (i.e., "the hemagglutininneuraminidase gene for PIV-1" of claim 1)

Parainfluenza 3 (i.e., "the 5' noncoding region of the PIV-3 fusion protein gene" of claim 1)

RSV (rsv 1)
Rsv 2
Rsv6
Rsv7
Rsv8 (i.e., "the F1 subunit of the fusion glycoprotein gene for RSV" of claim 1)

Mycoplasma pneumoniae for rRNA region
Mycoplasma pneumoniae for spacer region (i.e., the "16S rRNA sequence for *M. pneumoniae*" of claim 1)

Chlamydia pneumoniae for rRNA region
Chlamydia pneumoniae for spacer region (i.e., the "16S rRNA sequence for *C. pneumoniae*" of claim 1)

Bordotella pertussis
Bordatella parapertusis/bronchiseptica (i.e., the "at least one primer pair for the specific detection of *B. pertussis* and *B. parapertusis*" of claim 3).

The Commission appreciates that probes were elected for each of these organisms and has detailed the election on pages 2 and 3 of the Decision.

The applicants again elect these probes (i.e., the probes listed by the Commissioner on pages 2-3 of the Decision dated September 23, 2003), with traverse, for the purpose of being responsive to the Office Action of May 22, 2002.

As noted above, the Examiner required an election of a primer set for each organism from the primer sets of Tables 2 and 4.

The primer sets of Tables 2 and 4 however contain, for all but Mycoplasma pneumoniae, only one primer set for each organism. That is, once limited to only the primer sets of Tables 2 and 4, as required by the Examiner, the only choice of an election of a primer for any organism is an election of one of the two Mycoplasma pneumoniae forward primers (i.e., FP1 (SEQ ID NO: 17) or FP2 (SEQ ID NO:18)) of Table 4.

More specifically, the Commissioner and Examiner are urged to appreciate that Tables 2 and 4 of the specification describe, in total, the following 13 primer sets (wherein "FP" indicates a forward primer and "RP" indicates a reverse primer – see "*" in Table 4) which correspond to the 13 "pathogens" required by the Examiner and the Commissioner's Decision:

TABLE 2

Enterovirus primer set: ENTERO-FP1 (SEQ ID NO:35) and ENTERO-RP1 (SEQ ID NO:36);
Mycoplasma pneumoniae, when relating to 16S rRNA, primer set: MPN-FP1 (SEQ ID NO:37) and MPN-RP1 (SEQ ID NO:38);
Influenza A primer set: INFLUA-FP1 (SEQ ID NO:39) and INFLUA-RP1 (SEQ ID NO:40);
Influenza B primer set: INFLUB-FP1 (SEQ ID NO:41) and INFLUB-RP1 (SEQ ID NO:42);
Adenovirus primer set: ADENO-FP1 (SEQ ID NO:43) and ADENO-RP1 (SEQ ID NO:44);
Chlamydia pneumoniae, when relating to 16S rRNA, primer set: CPN-FP1 (SEQ ID NO:45) and CPN-RP1 (SEQ ID NO:46);
Parainfluenza 1 primer set: PIV1-FP1 (SEQ ID NO:47) and PIV1-RP1 (SEQ ID NO:48);
Parainfluenza 3 primer set: PIV3-FP1 (SEQ ID NO:49) and PIV3-RP1 (SEQ ID NO:50);
RSV primer set: RSV-FP1 (SEQ ID NO:51) and RSV-RP1 (SEQ ID NO:52);

TABLE 4

Mycoplasma pneumoniae, when relating to the spacer, primer set: FP1 (SEQ ID NO:17), FP2 (SEQID NO:18) and RP (SEQ ID NO:19);
Chlamydia pneumoniae, when relating to the spacer, primer set: FP (SEQ ID NO:20) and RP (SEQ ID NO:21);
Bordetella pertusis/Bordetella parapertussis primer set: FP (SEQ ID NO:22) and RP (SEQ ID NO:23).

As noted above, the only option with regard to the primer sets of Tables 2 and 4 is the selection of one of the two disclosed forward primers (i.e., FP) of the

Mycoplasma pneumoniae, when relating to the spacer, primer set (i.e., a choice between SEQ ID NO: 17 or SEQ ID NO: 18).

The applicants elected, with traverse, the primer of SEQ ID NO:18 in the Response of March 14, 2003 (see, page 3 of the Response), in response to a requirement for election of a single primer, and the applicants elected the primer set of SEQ ID NOs: 18 and 19, in the Response of June 24, 2002 (see, page 1 of the Response), in response to a requirement for election of a single primer set.

The applicants noted on page 3, second full paragraph, of the Response dated March 14, 2003, that the only choice with regard to primer pairs in Tables 2 and 4 of the application was with regard to Mycoplasma pneumoniae. Moreover, the applicants elected the primer set of SEQ ID NOs: 18 and 19 in the Response of June 24, 2002, because the above reiteration of Tables 2 and 4 was not believed to be required. Again, once the Examiner limited the applicants to the primer sets of Tables 2 and 4, the applicants reasonably believed that the Examiner, or anyone of ordinary skill in the art, would appreciate that the only choice relating to primer sets for each indicated pathogen was whether SEQ ID NO:17 or SEQ ID NO:18 was to be elected as the forward primer for the Mycoplasma pneumoniae primer set of Table 4.

Accordingly, the election of March 14, 2002 was not "incomplete" and the applicants did not "fail" to elect one set of primers for each of the required regions in the Response of June 24, 2002, as asserted by the Commissioner in the Decision. The applicants made the one election (i.e., one option) corresponding to the one choice available given the Examiner's restriction requirement and requirement to elected a primer set for each organism from the primer sets of Tables 2 and 4 of the application.

To the extent the Dismissal of the applicants Rule 181 Petition is based on these alleged inadequacies in the applicants Responses, the Decision dismissing the Rule 181 Petition is in error and the Decision should be reconsidered and vacated and a new Decision mailed which grants the applicants Rule 181 Petition and instructs the Examiner to examine all of the pending claims.

The applicants again elect, with traverse, the following primer sets in response to the Office Action dated May 22, 2002, as required by the Decision:

TABLE 2

Enterovirus primer set: ENTERO-FP1 (SEQ ID NO:35) and ENTERO-RP1 (SEQ ID NO:36);
Mycoplasma pneumoniae, when relating to 16S rRNA, primer set: MPN-FP1 (SEQ ID NO:37) and MPN-RP1 (SEQ ID NO:38);
Influenza A primer set: INFLUA-FP1 (SEQ ID NO:39) and INFLUA-RP1 (SEQ ID NO:40);
Influenza B primer set: INFLUB-FP1 (SEQ ID NO:41) and INFLUB-RP1 (SEQ ID NO:42);
Adenovirus primer set: ADENO-FP1 (SEQ ID NO:43) and ADENO-RP1 (SEQ ID NO:44);
Chlamydia pneumoniae, when relating to 16S rRNA, primer set: CPN-FP1 (SEQ ID NO:45) and CPN-RP1 (SEQ ID NO:46);
Parainfluenza 1 primer set: PIV1-FP1 (SEQ ID NO:47) and PIV1-RP1 (SEQ ID NO:48);
Parainfluenza 3 primer set: PIV3-FP1 (SEQ ID NO:49) and PIV3-RP1 (SEQ ID NO:50);
RSV primer set: RSV-FP1 (SEQ ID NO:51) and RSV-RP1 (SEQ ID NO:52);

TABLE 4

Mycoplasma pneumoniae, when relating to the spacer, primer set: FP2 (SEQ ID NO:18) and RP (SEQ ID NO:19);
Chlamydia pneumoniae, when relating to the spacer, primer set: FP (SEQ ID NO:20) and RP (SEQ ID NO:21);
Bordetella pertusis/Bordetella parapertussis primer set: FP (SEQ ID NO:22) and RP (SEQ ID NO:23).

Consideration of the following with regard to the Examiner's restriction requirement (i.e., point **(3)** above), is requested.

The Decision and the Office Action dated May 22, 2002, fail to indicate where the PCT rules relating to unity of invention allow for a separate requirement to elect one set of primers and a probe within the requirement to elect between methods (i.e., the Examiner's Group I) and "primers, probes and kits (i.e., the Examiner's Group II). The statement in the Decision that "typically only one set of primer [sic, primers] and probe combinations will be examined within a single patent application. See MPEP 803.01 which is directed to the examination of sets of molecules" (see, page 3, penultimate paragraph, of the Decision) is, with due respect, inadequate to support a finding of lack of unity of invention in a U.S. national phase of a PCT application.

Moreover, the Examiner's assertion in the Office Action dated May 22, 2002, that restriction between the subject matter of the Examiner's Group I and Group II is proper because "the single primers and probes are not required for use in any specific method and the kits do not require method steps, and alternatively, a probe, or a set of primers." is insufficient as a basis for requiring restriction in a U.S. national phase application of a PCT application. See, MPEP § 1850 and Annex B of the PCT Administrative Instructions, as further discussed below.

MPEP § 803.04, cited by the Commissioner in the Decision, states as follows, in relevant part (emphasis added):

> By statute, "[i]f two or more independent and distinct inventions are claimed in one application, the Commissioner may require the application to be restricted to one of the inventions." 35 U.S.C. 121. Pursuant to this statute, the rules

provide that "[i]f two or more independent and distinct inventions are claimed in a single application, the examiner in his action shall require the applicant . . . to elect that invention to which his claim shall be restricted." 37 CFR 1.142(a). See also 37 CFR 1.141(a).

Nucleotide sequences encoding different proteins are structurally distinct chemical compounds and are unrelated to one another. These sequences are thus deemed to normally constitute independent and distinct inventions within the meaning of 35 U.S.C. 121. Absent evidence to the contrary, each such nucleotide sequence is presumed to represent an independent and distinct invention, subject to a restriction requirement pursuant to 35 U.S.C. 121 and 37 CFR 1.141 et seq. Nevertheless, to further aid the biotechnology industry in protecting its intellectual property without creating an undue burden on the Office, the Commissioner has decided sua sponte to partially waive the requirements of 37 CFR 1.141 et seq. and permit a reasonable number of such nucleotide sequences to be claimed in a single application. See Examination of Patent Applications Containing Nucleotide Sequences, 1192 O.G. 68 (November 19, 1996).

It has been determined that normally ten sequences constitute a reasonable number for examination purposes. Accordingly, in most cases, up to ten independent and distinct nucleotide sequences will be examined in a single application without restriction. In addition to the specifically selected sequences, those sequences which are patentably indistinct from the selected sequences will also be examined. Furthermore, nucleotide sequences encoding the same protein are not considered to be independent and distinct inventions and will continue to be examined together.

In some exceptional cases, the complex nature of the claimed material, for example a protein amino acid sequence reciting three dimensional folds, may necessitate that the reasonable number of sequences to be selected be less than ten. In other cases, applicants may petition pursuant to 37 CFR 1.181 for examination of additional nucleotide sequences by providing evidence that the different nucleotide sequences do not cover independent and distinct inventions.

See MPEP § 1850 for treatment of claims containing independent and distinct nucleotide sequences in international applications filed under the Patent Cooperation Treaty (PCT) and national stage applications filed under 35 U.S.C. 371.

EXAMPLES OF NUCLEOTIDE SEQUENCE CLAIMS
Examples of typical nucleotide sequence claims impacted by the partial waiver of 37 CFR 1.141 et seq. (and the partial waiver of 37 CFR 1.475 and 1.499 et seq., see MPEP § 1850) include:

(A) an isolated and purified DNA fragment comprising DNA having at least 95% identity to a DNA sequence selected from SEQ ID Nos. 1-1,000;

(B) a combination of DNA fragments comprising SEQ ID Nos. 1-1,000; and

(C) a combination of DNA fragments, said combination containing at least thirty different DNA fragments selected from SEQ ID Nos. 1-1,000.

Applications claiming more than ten individual independent and distinct nucleotide sequences in alternative form, such as set forth in example (A), will be subject to a restriction requirement. Only the ten nucleotide sequences selected in response to the restriction requirement and any other claimed sequences which are patentably indistinct therefrom will be examined.

Applications claiming only a combination of nucleotide sequences, such as set forth in example (B), will generally not be subject to a restriction requirement. The presence of one novel and nonobvious sequence within the combination will render the entire combination allowable. The combination will be searched until one nucleotide sequence is found to be allowable. The order of searching will be chosen by the examiner to maximize the identification of an allowable sequence. If no individual nucleotide sequence is found to be allowable, the examiner will consider whether the combination of sequences taken as a whole renders the claim allowable.

> Applications containing only composition claims reciting different combinations of individual nucleotide sequences, such as set forth in example (C), will be subject to a restriction requirement. Applicants will be required to select one combination for examination. If the selected combination contains ten or fewer sequences, all of the sequences of the combination will be searched. If the selected combination contains more than ten sequences, the combination will be examined following the procedures set forth above for example (B). More specifically, the combination will be searched until one nucleotide sequence is found to be allowable with the examiner choosing the order of search to maximize the identification of an allowable sequence. The identification of any allowable sequence(s) will cause all combinations containing the allowed sequence(s) to be allowed.
>
> In applications containing all three claims set forth in examples (A)-(C), the Office will require restriction of the application to ten sequences for initial examination purposes. Based upon the finding of allowable sequences, claims limited to the allowable sequences as in example (A), all combinations, such as in examples (B) and (C), containing the allowable sequences and any patentably indistinct sequences will be rejoined and allowed.
>
> Rejoinder will be permitted for claims requiring any allowable sequence(s). Any claims which have been restricted and nonselected and which are limited to the allowable sequence(s) will be rejoined and examined.

The whole of MPEP § 803.04 therefore appears to relates to restriction of nucleotide sequences in applications other than U.S. national phase of PCT applications and appears to describe a partial waiver of the Commissioner's determination that each nucleotide sequence is an independent and distinct invention which define, in regular U.S. utility applications (i.e., non-U.S. national phase applications of PCT applications) a separately patentable invention. Without the partial

waiver of MPEP § 803.04, the Commissioner could require that each nucleotide sequence be pursued in separate divisional patent applications. The partial waiver MPEP § 803.04 permits a "reasonable number" of nucleotide sequences, indicated as "normally ten sequences", to be claimed in a single application. It has been the undersigned's experience that, in practice, this "waiver" is itself waived and the Patent Office requires restriction and examination of a single nucleotide sequence per application.

The Commissioner and the Exmainer are urged to appreciate that 37 CFR §§ 1.142(a) and 1.141(a) referred to in MPEP § 803.04 relate to the "independent and distinct" standard of restriction practice of U.S. utility applications, as opposed to the principles of unity of invention applied to U.S. national phase applications of PCT applications, such as the above-identified application, pursuant to 37 CFR § 1.499. The Commissioner and Examiner are urged to appreciate that 37 CFR § 1.499 provides as follows:

> 37 CFR § 1.499 Unity of invention during the national stage.
>
> If the examiner finds that a national stage application lacks unity of invention under § 1.475, the examiner may in an Office action require the applicant in the response to that action to elect the invention to which the claims shall be restricted. Such requirement may be made before any action on the merits but may be made at any time before the final action at the discretion of the examiner. Review of any such requirement is provided under §§ 1.143 and 1.144.

The only reference in MPEP § 803.04 to treatment of U.S. national phase applications of PCT applications are (a) a general direction to see MPEP § 1850, and (b) an example of claim types (generally dealing with large numbers of nucleotide

sequences) impacted by the partial waiver of 37 CFR § 1.499, as described in MPEP § 1850.

The claims of the present application are not believed to be characterized by the Example claim types (A)-(C) reproduced above from MPEP § 803.04 such that the requirement for the Patent Office to examine ten sequences in this specific situation is not believed to be applicable to the present application. The Commissioner and/or Examiner however is requested to advise the applicants if otherwise.

Accordingly, MPEP § 803.04 cited in the Decision instructs review of MPEP § 1850 for "treatment of claims containing independent and distinct nucleotide sequences ... in national stage applications filed under 35 U.S.C. 371."

MPEP § 1850 cited by MPEP §803.04, states as follows, in relevant part (emphasis added):

> 37 CFR 1.475 Unity of invention before the International Searching Authority, the International Preliminary Examining Authority and during the national stage.
>
> (a) An international and a national stage application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention"). Where a group of inventions is claimed in an application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.
>
> (b) An international or a national stage application containing claims to different categories of invention will be

considered to have unity of invention if the claims are drawn only to one of the following combinations of categories:

.....

THE REQUIREMENT FOR "UNITY OF INVENTION"

Any international application must relate to one invention only or to a group of inventions so linked as to form a single general inventive concept (PCT Article 3(4)(iii) and 17(3)(a), PCT Rule 3.1, and 37 CFR 1.475). Observance of this requirement is checked by the International Searching Authority and may be relevant in the national (or regional) phase.

The decision in Caterpillar Tractor Co. v. Commissioner of Patents and Trademarks, 231 USPQ 590 (E.D. Va. 1986) held that the Patent and Trademark Office interpretation of 37 CFR 1.141(b)(2) as applied to unity of invention determinations in international applications was not in accordance with the Patent Cooperation Treaty and its implementing regulations. In the Caterpillar international application, the USPTO acting as an International Searching Authority, had held lack of unity of invention between a set of claims directed to a process for forming a sprocket and a set of claims drawn to an apparatus (die) for forging a sprocket. The court stated that it was an unreasonable interpretation to say that the expression "specifically designed" as found in former PCT Rule 13.2(ii) means that the process and apparatus have unity of invention if they can only be used with each other, as was set forth in MPEP § 806.05(e).

Therefore, when the Office considers international applications as an International Searching Authority, as an International Preliminary Examining Authority, and during the national stage as a Designated or Elected Office under 35 U.S.C. 371, **PCT Rule 13.1 and 13.2 will be followed when considering unity of invention of claims of different categories without regard to the practice in national applications filed under 35 U.S.C. 111.** No change was made in restriction practice in United States national applications filed under 35 U.S.C. 111 outside the PCT.

In applying PCT Rule 13.2 to international applications as an International Searching Authority, an

International Preliminary Examining Authority and to national stage applications under 35 U.S.C. 371, examiners **should** consider for unity of invention all the claims to different categories of invention in the application and permit retention in the same application for searching and/or preliminary examination, claims to the categories which meet the requirements of PCT Rule 13.2.

PCT Rule 13.2, as it was modified effective July 1, 1992, no longer specifies the combinations of categories of invention which are considered to have unity of invention. Those categories, which now appear as a part of Annex B to the Administrative Instructions, has been substituted with a statement describing the method for determining whether the requirement of unity of invention is satisfied. Unity of invention exists only when there is a technical relationship among the claimed inventions involving one or more special technical features. The term "special technical features" is defined as meaning those technical features that define a contribution which each of the inventions considered as a whole, makes over the prior art. The determination is made based on the contents of the claims as interpreted in light of the description and drawings. Annex B also contains examples concerning unity of invention.

A. Independent and Dependent Claims

Unity of invention has to be considered in the first place only in relation to the independent claims in an international application and not the dependent claims. By "dependent" claim is meant a claim which contains all the features of another claim and is in the same category of claim as that other claim (the expression "category of claim" referring to the classification of claims according to the subject matter of the invention claimed, for example, product, process, use or apparatus or means, etc.).

If the independent claims avoid the prior art and satisfy the requirement of unity of invention, no problem of lack of unity arises in respect of any claims that depend on the independent claims. In particular, it does not matter if a dependent claim itself contains a further invention. Equally, no problem arises in the case of a genus/species situation where the genus claim avoids the prior art. Moreover, no problem arises in the case of a combination/subcombination

situation where the subcombination claim avoids the prior art and the combination claim includes all the features of the subcombination.

If, however, an independent claim does not avoid the prior art, then the question whether there is still an inventive link between all the claims dependent on that claim needs to be carefully considered. If there is no link remaining, an objection of lack of unity (that is, arising only after assessment of the prior art) may be raised. Similar considerations apply in the case of a genus/species or combination/subcombination situation.

This method for determining whether unity of invention exists is intended to be applied even before the commencement of the international search. Where a search of the prior art is made, an initial determination of unity of invention, based on the assumption that the claims avoid the prior art, may be reconsidered on the basis of the results of the search of the prior art.

B. Illustrations of Particular Situations

There are three particular situations for which the method for determining unity of invention contained in PCT Rule 13.2 is explained in greater detail:

(A) Combinations of different categories of claims; ...

Principles for the interpretation of the method contained in PCT Rule 13.2, in the context of each of those situations are set out below. It is understood that the principles set out below are, in all instances, interpretations of and not exceptions to the requirements of PCT Rule 13.2.

Examples to assist in understanding the interpretation on the three areas of special concern referred to in the preceding paragraph are set out below.

C. Combinations of Different Categories of Claims

The method for determining unity of invention under PCT Rule 13 shall be construed as permitting, in particular,

the inclusion of any one of the following combinations of claims of different categories in the same international application:

(A) In addition to an independent claim for a given product, an independent claim for a process specially adapted for the manufacture of the said product, and an independent claim for a use of the said product; or

(B) In addition to an independent claim for a given process, an independent claim for an apparatus or means specifically designed for carrying out the said process; or

(C) In addition to an independent claim for a given product, an independent claim for a process specially adapted for the manufacture of the said product and an independent claim for an apparatus or means specifically designed for carrying out the said process, it being understood that a process is specially adapted for the manufacture of a product if it inherently results in the product and that an apparatus or means is specifically designed for carrying out a process if the contribution over the prior art of the apparatus or means corresponds to the contribution the process makes over the prior art.

Thus, a process shall be considered to be specially adapted for the manufacture of a product if the claimed process inherently results in the claimed product with the technical relationship being present between the claimed product and claimed process. The words "specially adapted" are not intended to imply that the product could not also be manufactured by a different process.

Also an apparatus or means shall be considered to be specifically designed for carrying out a claimed process if the contribution over the prior art of the apparatus or means corresponds to the contribution the process makes over the prior art. Consequently, it would not be sufficient that the apparatus or means is merely capable of being used in carrying out the claimed process. **However, the expression specifically designed does not imply that the apparatus or means could not be used for carrying out another process, nor that the process could not be carried out using an alternative apparatus or means.**

...

PCT Rule 13.3 requires that the determination of the existence of unity of invention be made without regard to whether the inventions are claimed in separate claims or as alternatives within a single claim.

PCT Rule 13.3 is not intended to constitute an encouragement to the use of alternatives within a single claim, but is intended to clarify that the criterion for the determination of unity of invention (namely, the method contained in PCT Rule 13.2) remains the same regardless of the form of claim used.

PCT Rule 13.3 does not prevent an International Searching or Preliminary Examining Authority or an Office from objecting to alternatives being contained within a single claim on the basis of considerations such as clarity, the conciseness of claims or the claims fee system applicable in that Authority or Office.

LACK OF UNITY OF INVENTION
See Annex B of the Administrative Instructions for examples of unity of invention.

UNITY OF INVENTION - NUCLEOTIDE SEQUENCES

Under 37 CFR 1.475 and 1.499 et seq., when claims do not comply with the requirement of unity of invention, i.e., when the claimed subject matter does not involve "one or more of the same or corresponding special technical features," 37 CFR 1.475(a), an additional fee is required to maintain the claims in the same application. 37 CFR 1.476 (b).

The Commissioner has decided sua sponte to partially waive 37 CFR 1.475 and 1.499 et seq. to permit applicants to claim up to ten (10) nucleotide sequences that do not have the same or corresponding special technical feature without the payment of an additional fee. The PCT permits inventions that lack unity of invention to be maintained in the same international application for payment

> of additional fees. Thus, in international applications, for each group for which applicant has paid additional international search and/or preliminary examination fees, the USPTO has determined that up to four (4) such additional sequences per group is a reasonable number for examination. Further, claims directed to the selected sequences will be examined with claims drawn to any sequence combinations which have a common technical feature with the selected sequences. Nucleotide sequences encoding the same protein are considered to satisfy the unity of invention standard and will continue to be examined together.
>
> See MPEP § 803.04 for examples of nucleotide sequence claims impacted by this partial waiver of 37 CFR 1.475 and 1.499 et seq.

Accordingly, MPEP § 1850 states, with reference to the specific treatment of UNITY OF INVENTION - NUCLEOTIDE SEQUENCES and the "waiver" of MPEP § 803.04, that the threshold issue of unity of invention, i.e., finding that the claimed subject matter does not involve one or more of the same or corresponding special technical features, is still required and that if unity is not found in cases claiming nucleotide sequences, the Commissioner will allow examination of up to ten sequences without payment of additional fees. The reference in MPEP § 1850 to payment of additional fees is believed to be a reference to international applications where the USPTO is acting as the international preliminary examining authority, as opposed to any requirement relating to U.S. national phase (i.e., 37 CFR § 1.371) applications.

More importantly, unlike the statements in MPEP § 803.04 relating to treatment of nucleotide sequences in U.S. utility applications which are "deemed" to each define independent and distinct inventions, MPEP § 1850 does not similarly sua sponte define nucleotide sequences as lacking unity of invention. Accordingly, MPEP § 1805 is

believed to require the Patent Office, even in the case of claims to and involving nucleotide sequences, to establish a lack of unity of invention, such as by comparison to advances over the art, as described in MPEP § 1850 and Annex B of the PCT Administrative Instructions, to justify a restriction requirement.

The Examiner is requested therefore to demonstrate that the claims do not share the same or a corresponding special technical feature, as described, for example, in MPEP § 1850. The Examiner's assertion that the primers are not required in the claimed methods, as quoted above from the Office Action of May 22, 2002, is not understood. Clarification is requested in the event this justification for the restriction requirement is maintained. Moreover and more importantly, the Examiner is urged to appreciate that the recited sequences and their simultaneous use are a special technical feature of the claims which defines the invention over the art and demonstrates the existence of unity of invention. MPEP § 1850 and Annex B allow examination of claims defining separate categories, such as methods of using and products (as claimed in the present application), and the Examiner is requested to examine all the pending claims in the present application.

The Examiner has not demonstrated that the pending claims do not share the same or corresponding special technical feature, as described in MPEP § 1850, and the restriction requirement of May 22, 2002, therefore should be withdrawn, and all the claims examined on the merits.

Further, the applicants note that the pending claims define a process of using primers and, optionally, probes of the invention; the primers and probes *per se*; and a kit containing a set of primers of the invention and, optionally, probes of the invention.

Annex B of the Administrative Instructions states as follows:

> (a) Unity of Invention. Rule 13.1 deals with the requirement of unity of invention and states the principle that an international application should relate to only one invention or, if there is more than one invention, that the inclusion of those inventions in one international application is only permitted if all inventions are so linked as to form a single general inventive concept.
> (b) Technical Relationship. Rule 13.2 defines the method for determining whether the requirement of unity of invention is satisfied in respect of a group of inventions claimed in an international application. Unity of invention exists only when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" is defined in Rule 13.2 as meaning those technical features that define a contribution which each of the inventions, considered as a whole, makes over the prior art. The determination is made on the contents of the claims as interpreted in light of the description and drawings (if any).
> (c) Independent and Dependent Claims. Unity of invention has to be considered in the first place only in relation to the independent claims in an international application and not the dependent claims. By "dependent" claim is meant a claim which contains all the features of another claim and is in the same category of claim as that other claim (the expression "category of claim" referring to the classification of claims according to the subject matter of the invention claimed for example, product, process, use or apparatus or means, etc.).
> (i) If the independent claims avoid the prior art and satisfy the requirement of unity of invention, no problem of lack of unity arises in respect of any claims that depend on the independent claims. In particular, it does not matter if a dependent claim itself contains a further invention. Equally, no problem arises in the case of a genus/species situation where the genus claim avoids the prior art. Moreover, no problem arises in the case of a combination/subcombination situation where the subcombination claim avoids the prior art and the combination claim includes all the features of the subcombination.
> (ii) If, however, an independent claim does not avoid the prior art, then the question whether there is still an inventive

link between all the claims dependent on that claim needs to be carefully considered. If there is no link remaining, an objection of lack of unity a posteriori (that is, arising only after assessment of the prior art) may be raised. Similar considerations apply in the case of a genus/species or combination/subcombination situation.
(iii) This method for determining whether unity of invention exists is intended to be applied even before the commencement of the international search. Where a search of the prior art is made, an initial determination of unity of invention, based on the assumption that the claims avoid the prior art, may be reconsidered on the basis of the results of the search of the prior art.
(d) Illustrations of Particular Situations. There are three particular situations for which the method for determining unity of invention contained in Rule 13.2 is explained in greater detail:
(i) combinations of different categories of claims;
(ii) so-called "Markush practice"; and
(iii) intermediate and final products.
Principles for the interpretation of the method contained in

Rule 13.2, in the context of each of those situations are set out below. It is understood that the principles set out below are, in all instances, interpretations of and not exceptions to the requirements of Rule 13.2. Examples to assist in understanding the interpretation on the three areas of special concern referred to in the preceding paragraph are set out below.
(e) Combinations of Different Categories of Claims. The method for determining unity of invention under Rule 13 shall be construed as permitting, in particular, the inclusion of any one of the following combinations of claims of different categories in the same international application:
(i) in addition to an independent claim for a given product, an independent claim for a process specially adapted for the manufacture of the said product, and an independent claim for a use of the said product, or
(ii) in addition to an independent claim for a given process, an independent claim for an apparatus or means specifically designed for carrying out the said process, or
(iii) in addition to an independent claim for a given product, an independent claim for a process specially adapted for the manufacture of the said product and an independent claim for an apparatus or means specifically designed for carrying

> out the said process, it being understood that a process is specially adapted for the manufacture of a product if it inherently results in the product and that an apparatus or means is specifically designed for carrying out a process if the contribution over the prior art of the apparatus or means corresponds to the contribution the process makes over the prior art. Thus, a process shall be considered to be specially adapted for the manufacture of a product if the claimed process inherently results in the claimed product with the technical relationship being present between the claimed product and claimed process. The words "specially adapted" are not intended to imply that the product could not also be manufactured by a different process. Also an apparatus or means shall be considered to be "specifically designed for carrying out" a claimed process if the contribution over the prior art of the apparatus or means corresponds to the contribution the process makes over the prior art. Consequently, it would not be sufficient that the apparatus or means is merely capable of being used in carrying out the claimed process. However, the expression "specifically designed" does not imply that the apparatus or means could not be used for carrying out another process, nor that the process could not be carried out using an alternative apparatus or means.

Moreover, the following example of where unity exists between methods and products is provided in Annex B.

> Example 4
> Claim 1 Use of a family of compounds X as insecticides.
> Claim 2 Compound X1 belonging to family X.
> Provided X1 has the insecticidal activity and the special technical feature in claim 1 is the insecticidal use, unity is present.

In view of the above-quoted discussion and Example 4, for example, of Annex B, the applicants submit that the methods and products *per se*, including the kits containing the same, should be examined in a single application, as the claims are

submitted to satisfy the requirements of unity of invention and be patentable over the art.

Consideration of the following with regard to the responsiveness of the Amendment of March 10, 2003 (i.e., points **(4)** above), is requested, for completeness.

As noted above, the Commissioner has stated that the Examiner improperly withdrew the claimed methods of using primers other than SEQ ID NOs: 18 and 19 in the Office Action of September 10, 2002. The Amendment of March 10, 2003 was filed in response to the Office Action of September 10, 2002.

At the time the Amendment of March 10, 2003, was filed, the Examiner had withdrawn from consideration all primers except SEQ ID NOs: 18 and 19. Accordingly, absent some intervention from, for example, the Commissioner, the Examiner would have continued to consider only the primer set of SEQ ID NOs: 18 and 19, which are specific for Mycoplasma pneumoniae. The applicants believed therefore that it was likely that the Examiner was only examining claim 13 of the Amendment dated March 10, 2003, limited to the elected SEQ ID NO:18 and SEQ ID NO:19 primer set.

While the applicants continued to argue the inappropriateness of the restriction requirement, and believed the broader recitations of claim 13 of the Amendment dated March 10, 2003, should be examined in a single application, the applicants also wished to assure that at least one claim was directed to the subject matter indicated by the Examiner as still being under active consideration. Claim 19 therefore was introduced as being directed to the subject matter specifically indicated by the Examiner to be under active consideration. The Commissioner's confusion over the interpretation of

claim 19 was in fact created by the Examiner's withdrawal from consideration the subject matter of methods of using "other probes and primers ... as being directed to non-elected inventions." See, page 4 of the Decision. The Examiner's comment in the Office Action dated September 10, 2002 that "Applicant should also note that examination of the elected group is not limited in view of the required sequence election because the claims of group I will be examined in their broadest possible interpretation." only adds to the confusion in the record. The applicants are uncertain why restriction should be required if an election in response is not considered by the Examiner to limit examination. Clarification is requested. Claim 19 of the Amendment dated March 10, 2003 is not therefore confusing when taken in context and in response to the Office Action of September 10, 2002.

Accordingly, the Amendment of March 10, 2003, is submitted to have been completely responsive to the Office Action of September 10, 2002.

Reconsideration of the Decision is requested. Grant of the attached Renew Petition and withdrawal of the restriction requirement of May 22, 2002, and an Office Action on the merits of all the pending claims, are requested.

The Examiner is further requested to return of a completely initialed copy of the PTO 1449 Form filed March 13, 2001, pursuant to MPEP § 609, which lists, among other things, document DE 197 456. See, pages 6-7 of the Remarks of the Amendment filed March 10, 2003.

Specifically, the applicants note that the Information Disclosure Statement filed March 13, 2001, which lists, among other things, document DE 197 16 456, was filed in

compliance with the Rules. Specifically, the cited document was listed in the International Search Report from PCT/EP99/07065 and the relevance of the same is indicated as category "P" and "X". The International Search Report was filed with the Information Disclosure Statement and previously indicated as having been received by the Patent Office in the Notification of Acceptance dated September 14, 2001. A translation into the English language of the document should not be required. The Examiner is requested to appreciate in this regard that page 600-119 of the August 2001, copy of the MPEP indicates that "the Examiner will consider the documents cited in the International Search Report in a PCT National Stage application when the Form PCT/DO/EO/903 indicates that both the International Search Report and the copies of the documents are present in the National Stage file". Form PCT/DO/EO/903 is the Notification of Acceptance referred to above.

The Examiner is also requested to see page 600-122 of the August 2001 version of the MPEP which indicates as follows:

> Where the information listed [in a PTO-1449 Form] is not in the English language, but was cited in a Search Report or other action by foreign patent office in a counterpart foreign application, the requirement for a concise explanation of relevance can be satisfied by an English language version of the Search Report or Action which indicates the degree of relevance found by the foreign office. This may be an explanation of which portion of the reference is particularly relevant, to which claims it applies, or merely an "X", "Y", or "A" indication on a Search Report.

Accordingly, nothing further should be required. For completeness however attached is an English language translation of the cited DE 197 16 456. Return of an

initialed copy of the previously submitted PTO-1449 Form, indicating consideration of the listed document, or return of a PTO 892 Form listing the same, is requested.

The Examiner is requested to contact the undersigned in the event anything further is required for grant of the attached Renewed Petition and/or in response to the Office action dated May 22, 2002.

The claims are submitted patentable over the cited art.

Grant of the attached Renewed Petition Under Rule 181 and a Notice of Allowance are requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____
B. J. Sadoff
Reg. No. 36,663

BJS
1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

Method for detecting microorganisms, in particular those causing infectious diseases

Method for detecting microorganisms, in particular those causing infectious diseases, by means of simultaneous amplification of a plurality of target sequences in a reaction vessel, in a so-called multiplex PCR method, by contacting a patient's sample with a primer solution consisting of a plurality of primers, initiating a polymerase chain reaction (PCR), where, for disease-causing microorganisms which have only one RNA, part of the patient's sample is subjected to a preceding reverse transcription, and then the PCR products are subjected to a digoxigenin labelling with probes chosen appropriate for the primers, and subsequently obtaining information about the presence of a microorganism belonging to the primer by capture probe analysis and/or photometric analysable change in the colour value of the sample.

Description

The invention relates to a method for detecting microorganisms, in particular those causing infectious diseases.

Microorganisms causing infectious diseases, in particular those of the human respiratory tract, may cause diseases with particularly severe courses especially in weakened patients (e.g. with cardiovascular disorders or with cancer). However, even "normal" coryzal illnesses are the commonest of all human illnesses. The loss of work and money caused thereby in the Federal Republic of Germany alone each year reaches astronomical figures. They are caused by various microorganisms which induce only a comparatively weak defence in the body, for which

reason such infections can occur repeatedly. At least some of these disease-causing microorganisms can now be treated with antibiotics, although it is unfortunately not at present known how these disease-causing microorganisms can be distinguished from one another solely on the basis of the patients' symptoms. The methods known to date for detecting microorganisms causing coryzal illnesses are extremely complicated, time-consuming and costly.

In the first place, the practicability of culturing is low because the result is available only after a long time, at least days, mostly weeks, and culturing is extremely elaborate and therefore cost-intensive and, last but not least, has low sensitivity; the average paediatric hospital stay is 3 to 5 days so that the patient has usually returned home when the disease-causing microorganism is detected. Culturing the disease-causing microorganisms is therefore useless for acute patient care.

Serology, which is also known and in which an increase in the antibodies against a disease-causing microorganism in the patient is investigated, is likewise conditional on a very long run-up time. It is necessary to compare two blood samples from the patient which have been taken at an interval of 3 to 4 weeks.

For care of the patient it is crucially important to employ the drug which is correct for the particular disease-causing microorganism or, where appropriate, no antibiotic at all. A further necessity in the hospital is that various isolation measures must be taken, depending on the microorganism which has infected the patients, such as, for example, the cohorting of patients with one microorganism in one room, who are then cared for only by one member of the nursing staff. It is therefore very important that the particular disease-causing microorganism is recognized quickly.

The object on which the invention is based is to provide a rapid method for detecting the microorganisms which come under consideration.

This is achieved according to the invention by the features of the main claim. The dependent claims represent advantageous embodiments of the invention.

It is possible with the aid of the described polymerase chain reaction (PCR) for the first time also to detect rapidly and reliably microorganisms which cause disease in the respiratory tract. In this case it is possible to amplify a plurality of target sequences in one reaction vessel simultaneouly (multiplex PCR). This makes it possible to undertake epidemiological investigations in order to find out which microorganisms cause respiratory tract diseases in which population, and how frequently, and to inform the treating physician within one working day of whether the patient will profit from treatment with a particular antibiotic or not. It was not possible in the past to make this decision appropriately because no objective aid to decision was available.

The diagnostic multiplex PCR used for this purpose makes it possible, by contrast, to make a rapid decision concerning the administration of antibiotics on the basis of the microorganisms found. For example, infectious diseases caused by mycoplasmas or chlamydias can be treated with a macrolide antibiotic, whereas there is as yet no therapy for disease-causing viruses. If no disease-causing microorganism is detected, a bacterium must be suspected as the cause, depending on the clinical findings, in which case treatment with a beta-lactam antibiotic (pneumococci, haemophilus) is necessary.

The problem which occurs in particular in the implementation, of combining different primers in a multiplex PCR, is solved by the primers detailed in the dependent claims.

In this connection, the chosen selection of at least one of the primers

- Chlamydia pneumoniae
CpnA 5'-TGA CAA CTG TAG AAA TAC AGC-3'
CpnB 5'-CGC CTC TCT CCT ATA AAT-3'; and
- Mycoplasma pneumoniae
MP1 5'-AAG GAC CTG CAA GGG TTC GT-3'
MP2 5'-CTC TAG CCA TTA CCT GCT AA-3'

provides an indicator of whether treatment is necessary with a macrolide antibiotic, and the total number of nine different primers CpnA,-B; MP1,-2; EV1,-2; RSV1,-2; InfA NS1, NS2; InfB NS1, NS2; Adh1,-2; PIV1 1,2; PIV3 1,3 covers all the usual coryzal illnesses, so that if there is a negative finding it can be concluded that the cause is bacterial.

Although finding only viral microorganisms affords no direct pointer to therapy, epidemiological investigations and appropriate cohorting with patients infected by the same virus become possible, and hospital-acquired infections can be avoided.

Further features and advantages of the invention are evident from the following description of a preferred sample preparation and the performance of the method with suitable primers and probes.

In a sample preparation initially used for the experiments, for example, 100 µl of nasopharyngeal discharge are diluted with 100 µl of 0.9% NaCl solution and extracted with 1 volume of phenol/chloroform/isoamyl alcohol in a composition of

25:24:1 in a final concentration of 0.1% SDS. After centrifugation in a bench centrifuge for 5 minutes, the upper aqueous phase is aspirated off, extracted with chloroform/isoamyl alcohol 24:1 and centrifuged for 2 min. The nucleic acids in the supernatant are precipitated with 2.5 volumes of ethanol at a final concentration of 0.3 M sodium acetate for 5 min at -70°C, and spun down for 20 min. The pellet is taken up in 15 µl of diethyl pyrocarbonate-treated double-distilled H_2O.

Then 5 µl of this undergoes reverse transcription in a 20 µl reaction mixture with 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, in each case 1 mM dATP, dCTP, dTTP and dGTP (e.g. from Pharmacia), 0.2 µg/µl hexanucleotide mix (e.g. from Boehringer Mannheim), 20 U of RNAsin (e.g. Promega) and 10 U of Mu-MLV reverse transcriptase (e.g. Eurogentec) (final concentrations in each case) at 37°C for 60 min. After thermal inactivation of the enzyme at 90°C for 5 min, the 20 µl are employed in the multiplex PCR.

The polymerase chain reaction takes place in an 80 µl reaction mixture with 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, in each case 0.2 mM dATP, dCTP, and dGTP, 0.19 mM dTTP, 0.01 mM digoxigenin-11-dUTP (Boehringer Mannheim), 1 µM of each primer solution (see below) and 5 U of ampliTaq Gold polymerase (Perkin-Elmer).

The PCR reaction takes place in the thermoblock of a PCR thermocycler (PE 9600 Perkin Elmer) after an initial 10-minute denaturation at 94°C using a temperature profile with a total of 40 cycles each consisting of denaturation at 94°C for 30 sec, hybridization of the primers at 50°C and DNA synthesis at 72°C, and a final 7-minute incubation at 72°C.

A PCR ELISA (Boehringer) follows to differentiate the PCR products. The digoxogen-labelled amplicons are immobilized with the aid of specific biotin-labelled probes onto streptavidin-coated microtitre plates. The bound hybrid can be detected with an anti-dioxygenin-peroxidase conjugate and a colour substrate.

For this purpose, in each case 25 µl of denaturation solution (0.2N NaOH, 0.1% SDS) and 5 µl of a PCR product are placed in the wells of the streptavidin-coated microtitre plates. 9 wells are charged for each PCR product. Denaturation for 10 minutes is followed by addition of the 9 different hybridization solutions which are composed of the particular biotinylated probe (final concentration 7.5 pmol/ml; see below for sequences) and hybridization buffer (Boehringer Mannheim).

After shaking at 37°C for 1 hour, the plate is washed three to five times with washing solution, and 200 µl of an anti-dig-peroxidase conjugate is placed in each well. After shaking at 37°C for 30 min, washing is repeated and the colour substrate ABTS is pipetted into the wells, and after shaking at 37°C for 30 min the change in colour can be measured photometrically in an ELISA reader at 405 nm. A 490 nm reference filter is used. Thus, colour changes occur only in the wells in which, owing to the probes, PCR product and the anti-dig peroxidase adhere to streptavidin.

The appended list indicates the primers used and the probes suitable therefor which were used:

Primers used:

Enterovirus:- EV1 5'-ATT GTC ACC ATA AGC AGC CA-3'
EV2 5'-TCC TCC GGC CCC TGA ATG CG-3'

Mycoplasma pneumoniae: MP1 5'-AAG GAC CTG CAA GGG TTC GT-3'
MP2 5'-CTC TAG CCA TTA CCT GCT AA-3'

Influenzavirus type A: InfA NS1 5'-AAG GGC TTT CAC CGA AGA GG-3'
InfA NS2 5'-CCC ATT CTC ATT ACT GCT TC-3'

Influenzavirus type B: InfB NS1 5'-ATG GCC ATC GGA TCC TCA AC-3'
InfB NS2 5'-TGT CAG CTA TTA TGG AGC TG-3'

Adenovirus: Adh1 5'-GCC GAG AAG GGC GTG CGC AGG TA-3'
Adh2 5'-ATG ACT TTT GAG GTG GAT CCC ATG GA-3'

Chlamydia pneumoniae: CpnA 5'-TGA CAA CTG TAG AAA TAC AGC-3'
CpnB 5'-CGC CTC TCT CCT ATA AAT-3'

Parainfluenzavirus type 1:
PIV1 1 5'-CAC ATC CTT GAG TGA TTA AGT TTG ATG A-3'
PIV1 2 5'-ATT TCT GGA GAT GTC CCG TAG GAG AAC-3'

Perainfluenzavirus type 3:
PIV3 1 5'-TAG CAG TAT TGA AGT TGG CA-3'
PIV3 2 5'-AGA GGT CAA TAC CAA CAA CTA-3'

Respiratory Syncytial Virus (RSV):
RSV1 5'-TGT TAT AGG CAT ATC ATT GA-3'
RSV 2 5'-TTA ACC AGC AAA GTG TTA GA-3'

Probes (3'-end biotinylated):

Sonden (3'-Ende biotinyliert):

Enterovirus: EV3 5'-GAA ACA CGG ACA CCC AAA GTA-3'

Mycoplasma pneumoniae: MP3 5'-ACT CCT ACG GGA GGC AGC AGT A-3'

Influenzavirus type A: InfA3 5'-GTC CTC ATC GGA GGA CTT GAA TGGAATGAT-3'

Influenzavirus type B: InfB3 5'-GTC AAG AGC ACC GAT TAT CAC C-3'

Adenovirus: Adh 3: 5'-CTC GAT GAC GCC GCG GTG C-3'

Chlamydia pneumoniae: CpnC 5'-TCT TGC TAC CTT CTG TAC TAA C-3'

Parainfluenzavirus type 1: PIV1C 5'-TAC CTT CAT TAT CAA TTG GTA AGT CAA TAT ATG -3'

Parainfluenzavirus type 3: PIV3C 5'-AAA ATT CCA AAA GAG ACC GGC -3'

RSV: RSV3 5'-TAC ACC TGC ATT AAC ACT AA-3'

It would also be advantageous to arrange, with the method of reserve hybridization, immobilized probes in separate regions on a support material, onto which each PCR product would then be put only for the evaluation.

Patent claims

1. Method for detecting microorganisms, in particular those causing infectious diseases, characterized by
   - a simultaneous amplification of a plurality of target sequences in a reaction vessel, a so-called multiplex PCR, in which a patient's sample is contacted with a primer solution consisting of a plurality of primers, and a polymerase chain reaction (PCR) is initiated,
   - where, for disease-causing microorganisms having only one RNA, part of the patient's sample is subjected to a preceding reverse transcription,
   - and then the PCR products are subjected to a digoxigenin labelling with probes chosen appropriate for the primers,
   - and subsequently to obtain information about the presence of a microorganism belonging to the primer by capture probe analysis and/or photometric analysable change in the colour value of the sample.

2. Method for detecting microorganisms according to Claim 1, characterized in that a Mycoplasma pneumoniae MP1, MP2 and Chlamydia pneumoniae CpnA, CpnB are used as primers to identify macrolide antibiotic-sensitive microorganisms.

3. Method for detecting microorganisms according to either of the preceding claims, characterized in that an RSV primer (respiratory syncytial virus) RSV1, RSV2 is used.

4. Method for detecting microorganisms according to any of the preceding claims, characterized in that an enterovirus primer EV1, EV2 is used.

5. Method for detecting microorganisms according to any of the preceding claims, characterized in that influenza virus primers type A and type B InfA NS1, InfA NS2 and InfB NS1, InfB NS2 are used.

6. Method for detecting microorganisms according to any of the preceding claims, characterized in that an adenovirus primer Adh 1 5'-GCC GAG AAG GGC GTG CGC AGG TA-3', Adh2 5'-ATG ACT TTT GAG GTG GAT CCC ATG GA-3' is used.

7. Method for detecting microorganisms according to any of the preceding claims, characterized in that parainfluenza virus primers type 1 PIV1 1, PIV1 2 and type 3 PIV3 1, PIV3 2 are used.

8. Method for detecting all therapeutically relevant microorganisms of the respiratory tract, characterized in that the primers

Enterovirus: EV1 5'-ATT GTC ACC ATA AGC AGC CA-3'
EV2 5'-TCC TCC GGC CCC TGA ATG CG-3'

Mycoplasma pneumoniae: MP1 5'-AAG GAC CTG CAA GGG TTC GT-3'
MP2 5'-CTC TAG CCA TTA CCT GCT AA-3'

Influenzavirus type A: InfA NS1 5'-AAG GGC TTT CAC CGA AGA GG-3'
InfA NS2 5'-CCC ATT CTC ATT ACT GCT TC-3'

Influenzavirus type B: InfB NS1 5'-ATG GCC ATC GGA TCC TCA AC-3'
InfB NS2 5'-TGT CAG CTA TTA TGG AGC TG-3'

Adenovirus: Adh1 5'-GCC GAG AAG GGC GTG CGC AGG TA-3'
Adh2 5'-ATG ACT TTT GAG GTG GAT CCC ATG GA-3'

Chlamydia pneumoniae: CpnA 5'-TGA CAA CTG TAG AAA TAC AGC-3'
CpnB 5'-CGC CTC TCT CCT ATA AAT-3'

Parainfluenzavirus type 1:
PIV1 1 5'-CAC ATC CTT GAG TGA TTA AGT TTG ATG A-3'
PIV1 2 5'-ATT TCT GGA GAT GTC CCG TAG GAG AAC-3'

Parainfluenzavirus type 3:
PIV3 1 5'-TAG CAG TAT TGA AGT TGC CA-3'
PIV3 2 5'-AGA GGT CAA TAC CAA CAA CTA-3'

Respiratory Syncytial Virus (RSV):
RSV1 5'-TGT TAT AGG CAT ATC ATT GA-3'
RSV 2 5'-TTA ACC AGC AAA GTG TTA GA-3'

are put in combination with one another after reverse transcription, where appropriate, of part of the patient's samples into a multiplex PCR, and

then hybridization solutions biotinylated at the 3′ end

Probes (3′ end biotinylated):

Sonden (3'-Ende biotinyliert):

Enterovirus: EV3 5'-GAA ACA CGG ACA CCC AAA GTA-3'

Mycoplasma pneumoniae: MP3 5'-ACT CCT ACG GGA GGC AGC AGT A-3'

Influenzavirus type A: InfA3 5'-GTC CTC ATC GGA GGA CTT GAA TGGAATGAT-3'

Influenzavirus type B: InfB3 5'-GTC AAG AGC ACC GAT TAT CAC C-3'

Adenovirus: Adh 3: 5'- CTC GAT GAC GCC GCG GTG C-3'

Chlamydia pneumoniae: CpnC 5'-TCT TGC TAC CTT CTG TAC TAA C-3'

Parainfluenzavirus type 1: PIV1C 5'-TAC CTT CAT TAT CAA TTG GTA AGT CAA TAT ATG -3'

Parainfluenzavirus type 3: PIV3C 5'-AAA ATT CCA AAA GAG ACC GGC -3'

RSV: RSV3 5'-TAC ACC TGC ATT AAC ACT AA-3'

are used as probes.